Conclusions: *BRAF* V600E mutation in the primary tumor marks a subgroup of patients with especially poor prognosis and indicates that this patients' population might justify foregoing approved treatments in favor of investigational therapy. The adverse significance of BRAF mutation should be used for patient selection and stratification in future clinical trials. These results underscore the potential of mutational profiling to help patients whose tumors have a different natural history or differential response to traditional therapies.

1017 POSTER DISCUSSION

Impact of sequence variants in CYP2C8 on paclitaxel clearance in ovarian cancer patients

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Background: Toxicity and effect of paclitaxel vary greatly between patients and remain a clinically relevant problem with regard to the handling of dose delay/reduction or termination of treatment. We investigated the notion that single nucleotide polymorphisms (SNPs) in *CYP2C8* could be partly responsible for this variation. Paclitaxel is mainly metabolized by CYP2C8; SNPs have been investigated in this context before but conclusions are still lacking. We present early results from a prospective study of paclitaxel clearance (CL) in 93 Caucasian females with ovarian cancer with regard to the *CYP2C8* *1b, *1c, *3 and *4 genotypes. Ten other genes are considered
Materials and Methods: All patients were diagnosed with primary ovarian/peritoneal cancer and received 175 mg/m^2 paclitaxel over 3 hrs plus carboplatin AUC5−6 q3w. All patients gave written and verbal consent. The study was approved by ethics commitees in Denmark and Sweden. Blood was sampled at 3, 5−8 and 18−24hrs after start of infusion. Total plasma paclitaxel was quantified by HPLC. CremophorEL (CrEL) was determined using Coomassie blue assay. CL of unbound paclitaxel was estimated using total concentrations, CrEL and other parameters in the model described by Henningsson et al.in 2003 using NONMEM VI. Genotyping were done using Pyrosequencing. Genotypes were in HW equilibrium, except *1b ($p = 0.01$).
Results: The PK model predicted the data well. The CL of unbound paclitaxel was lower for patients with the *CYP2C8*3* and **4* variants ($p < 0.05$). For the one patient carrying both *3 and *4 variant CL was 269.7 l/h.

CYP2C8 variant	N	Paclitaxel CL geometric mean (l/h)	95% c.i	P-value (T-test of log transformed CL)
Wt/Wt	49	395	370.3–421.4	
Wt/*1b + *1b/*1b (n = 1)	44	374.5	348.8–402.2	0.267
Wt/Wt	69	382.4	362.1–404	
Wt/*1c	24	393.1	355.4–434.8	0.617
Wt/Wt	74	394.7	375.3–415.3	
Wt/*3	19	350	310–395.3	0.041*
Wt/Wt	86	390.9	372.1–410.7	
Wt/*4	7	320.8	281.7–365.3	0.028*

Conclusions: This study implies reduced elimination of paclitaxel in Caucasian female patients with the *CYP2C8*3* and **4* genotypes. This confirms several *in vitro* studies and pilot studies but is different to Henningsson et al 2003 and Marsh et al 2006 which could be explained by differences in dose ranges, infusion times and/or related to gender. The finding is important in terms of understanding inter individual variability of paclitaxel pharmakokinetics and might in the future provide useful information for individualized chemotherapy.

1018 POSTER DISCUSSION

Trichostatin A and decitabine reverses resistance to docetaxel in human breast cancer cells

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Introduction: Resistance mechanisms of the chemotherapeutic docetaxel are poorly understood, however, epigenetic modifications may be important. Epigenetic events such as DNA methylation and histone deacetylation can alter gene expression due to gene silencing. Altered gene expression can be linked to drug resistance, therefore, we hypothesise that treatment with a DNA methylation or a histone deacetylation inhibitor may reverse docetaxel resistance in human breast cancer cells.
Methods: Docetaxel-resistant human breast cancer cells (MCF-7 and MDA-MB-231) were treated with either trichostatin A (TSA), a histone deacetylation inhibitor, or 5-aza-2′-deoxycytidine (decitabine), a DNA methylation inhibitor, or in combination. Global methylation and DNA methyltransferase (DNMT) enzyme activity was measured using an ELISA-based assay. Histone acetylation levels were measured by western blot analysis. Quantitative PCR was used to measure *DNMT* gene expression changes. Cell viability was measured using MTT assay.
Results: Decitabine did not alter response to docetaxel in either cell line even though global methylation, DNMT enzyme activity and *DNMT* gene expression were changed following treatment with decitabine. Histone H3 acetylation was increased after TSA treatment. Addition of 200 nM TSA increased sensitivity to docetaxel in MCF-7 resistant cells ($P = 0.007$) but, in contrast, increased resistance to docetaxel in MDA-MB-231 cells. Concurrent treatment with decitabine and TSA enhanced response to docetaxel in MCF-7 docetaxel-resistant cells (IC_{50} resistant cells: 18.1 ± 1.9 vs. IC_{50} resistant-treated cells: 5.5 ± 5.0; $P = 0.016$). Furthermore, docetaxel sensitivity was also increased in MDA-MB-231 cells (IC_{50} resistant cells: 43.3 ± 8.6 vs. IC_{50} resistant-treated cells: 22.4 ± 2.6; $P = 0.016$).
Conclusions: Decitabine alters the methylation machinery in both docetaxel-resistant cell lines, but response to docetaxel could not be enhanced using this DNA methylation inhibitor. Trichostatin A did increase sensitivity to docetaxel but only in MCF-7 cells suggesting a cell line specific effect using this histone deacetylation inhibitor. Treatment with both inhibitors, however, increased response to docetaxel in both docetaxel-resistant cell lines. These results suggest that combination of methylation and histone deacetylation inhibitors may offer better therapeutic potential than using single inhibitors to overcome drug resistance.

1019 POSTER DISCUSSION

Withaferin-A modulates Akt/FOXO3a/Par-4 axis and induces apoptosis in human prostate cancer cells

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Background: In advanced prostate cancer (PCa), the most common apoptotic genes, such as p53 and PTEN, are either mutated or deleted; such mutations render PCa resistant to current treatments. To overcome this therapeutic impasse, activation of other pro-apoptotic signaling pathways is necessary. The forkhead transcription factor class O (FOXO) family regulates a wide variety of cellular responses, several of which are related to important aspects of tumorigenesis, and activation of FOXO 3a results in either cell cycle arrest or apoptosis. Akt, which negatively regulates FOXO 3a, is highly expressed in androgen-independent prostate cancer (AIPC) cells and thereby blocks the pro-apoptotic function of FOXO 3a. Logically, overcoming this block might be an effective chemopreventive and/or chemotherapeutic strategy.
Materials and Methods: Differential expression of Akt/FOXO 3a/Par-4 expressions in AIPC cells were studied in human PCa cells. AIPC cells were exposed to WA was studied with transwell migration and Matrigel invasion assays. To study the effects on AKT/FOXO 3a/Par-4 signaling were determined by SDS-PAGE, Western blot; Par-4 expression was evaluated after overexpression Akt and FOXO 3a by RT-PCR. Knock down and over expression of FOXO 3a and Akt on Par-4 gene was studied using siRNA and plasmid transfections, respectively.
Results: We have identified a dietary molecule, Withaferin-A, that in AIPC cells concomitantly inhibits Akt phosphorylation and activates FOXO 3a by importing it into the nucleus to induce apoptosis. Similarly, activation and nuclear import of Par-4 induce apoptosis. Interestingly, over expression of Akt inhibits the activation of both FOXO 3a and Par-4, causing PCa to become resistant to WA treatment. Based on these observations, we hypothesize FOXO 3a and Par-4 signaling directly interacts, as inhibition of FOXO 3a downregulates Par-4, as well as WA-induced apoptosis in AIPC cells. Notably, inhibition of Par-4 does not alter WA-induced FOXO 3a activation. This suggests FOXO 3a is upstream of Par-4 signaling, and that FOXO 3a signaling is essential for WA-induced, Par-4-mediated apoptosis in AIPC cells. Our data reveal that over expression of FOXO 3a up-regulates Par-4 expression, and that FOXO 3a physically binds Par-4 and activates Par-4 at the promoter level.
Conclusions: Overall, our studies indicate that Par-4 is one of the target genes of FOXO 3a, and that activation of Par-4 signaling by WA leads to apoptosis in AIPC cells.